AVIAN DISEASES 50:15–21, 2006

Antigenic and Genetic Characterization of an Avian Poxvirus Isolated from an Endangered Hawaiian Goose (*Branta sandvicensis*)

Taejoong Kim[A] and Deoki N. Tripathy[B]

Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Illinois at Urbana–Champaign, 2001 S. Lincoln Avenue, Urbana, IL 61802

Received 29 June 2005; Accepted 1 September 2005

SUMMARY. An avian poxvirus from cutaneous lesions in a Hawaiian goose (*Branta sandvicensis*) was characterized in this study. The virus was isolated by inoculation onto the chorioallantoic membranes (CAMs) of developing chicken embryos. Cytoplasmic inclusion bodies were observed on histopathological examination of CAM lesions. Western blotting analysis using polyclonal antiserum against fowl poxvirus (FWPV) showed differences from FWPV, but a similar antigenic profile between Hawaiian goosepox (HGP) isolate and two previous Hawaiian poxvirus isolates were observed. Still three avian poxviruses from Hawaiian birds showed distinguishable reaction in approximately 27, 34, 35, and 81 kDa proteins when polyclonal antibodies against the Hawaiian poxvirus isolate (Alala/lanakila) were used. Restriction fragment length polymorphisms (RFLP) of DNA of this isolate also showed differences from those of FWPV and previous avianpox isolates from Hawaiian forest birds. While nucleotide sequences of a 5.3-kb *Pst*I-*Hin*dIII fragment of the genome of HGP isolate revealed very high homology (99% identities) with Canary poxvirus (CNPV) ORF266-274, and like CNPV, homologs of three FWPV ORFs (199, 200, and 202) including any reticuloendotheliosis virus (REV) sequences are not present in the genome of HGP isolate.

RESUMEN. Caracterización genética y antigénica de un virus de viruela aviar aislado a partir de un ganso Hawaiano (*Branta sandvicensis*) en peligro de extinción.

Se caracterizó un virus de viruela aviar aislado a partir de lesiones cutáneas presentes en un ganso Hawaiano (*Branta sandvicensis*). El virus se aisló mediante la inoculación de la muestra en la membrana corioalantoidea de embriones de pollo. Al examen histopatológico, se observó la presencia de cuerpos de inclusión intracitoplasmáticos en las lesiones presentes en la membrana corioalantiodea. Mediante el análisis de inmunotransferencia puntual Western empleando anticuerpos policlonales contra el virus de viruela aviar, se observaron diferencias con el virus de viruela aviar, sin embargo, se observó un perfil antigénico similar entre el virus de viruela del ganso Hawaiano y dos virus de viruela Hawaianos aislados con anterioridad. Se observaron reacciones diferentes en cuatro proteínas de aproximadamente 27, 34, 35 y 81-kD al comparar tres virus de viruela de aves Hawaianas empleando anticuerpos policlonales contra el aislamiento de viruela aviar Hawaiano (Alala/lanakila). El análisis de la longitud de los fragmentos de restricción del ADN del virus de viruela del ganso Hawaiano mostró diferencias al ser comparado con el virus de viruela aviar y con otros virus de viruela aviar aislados con anterioridad a partir de aves silvestres Hawaianas. Mientras la secuencia de nucleótidos del fragmento *Pst*I-*Hind*III de 5.3-kb del genoma del virus de viruela del ganso Hawaiano mostró una alta similitud (99%) con el virus de viruela de los canarios en el marco abierto de lectura (posiciones 266 a la 274), y al igual que el virus de viruela de los canarios, secuencias homólogas de tres marcos abiertos de lectura (199, 200 y 202), incluyendo cualquiera de las secuencias del virus de la reticuloendoteliosis, no se encuentran presentes en el genoma del aislamiento del virus de viruela del ganso Hawaiano.

Key words: Hawaiian goosepox isolate, fowl poxvirus, canary poxvirus, antigenic and genetic characterization

Abbreviations: CAM = chorioallantoic membrane; CNPV = canary poxvirus; FWPV = fowl poxvirus; HGP = Hawaiian goosepox; LTR = long terminal repeat; ORF = open reading frame; PBS = phosphate buffered saline; PCR = polymerase chain reaction; PVDF = polyvinylidinefluoride; REV = reticuloendotheliosis virus; RFLP = restriction fragment length polymorphisms; VGPV = *Vultur gryphus* poxvirus

Poxvirus infection has been considered one of important extinction factors for the endangered avian species in Hawaii. In this regard many cases of natural poxvirus infection in endangered avian species have been reported in North America (3,21). Although, in these cases, the clinical history supported by histopathologic and/or electron microscopic observation was reported, antigenic or genetic information about the causative agents has been very limited (19).

Fowl poxvirus (FWPV), type species of the genus *Avipoxvirus* that affects chickens and turkeys, has been studied in detail because of its economic importance for commercial poultry (18). Currently, 10 species (designated as canary poxvirus, fowl poxvirus, junco poxvirus, mynah poxvirus, pigeon poxvirus, psittacine poxvirus, quail poxvirus, sparrow poxvirus, starling poxvirus, and turkey poxvirus) are listed as members of the genus *Avipoxvirus* (7). To differentiate these virus species, host-susceptibility and/or cross-protection studies in poultry have been used. Although avian poxviruses possess cross-reacting antigens, information about their genetic and antigenic differences has been limited (4,8).

Earlier, two avian poxviruses (Alala/lanakila and Apapane/4263-219) isolated from Hawaiian forest birds showed distinct biologic and genetic characteristics (19). Similarly, *Vultur gryphus* poxvirus (VGPV), an avian poxvirus isolated from Andean condor, showed unique genetic, antigenic, and biological properties distinguishing it from FWPV (5).

Because information about the poxviruses that infect endangered Hawaiian birds and their relationship with those that infect domestic birds is not available, the objectives of this study were a) antigenic comparison of Hawaiian goosepox (HGP) isolate with FWPV and two previous poxvirus isolates from Hawaiian birds by Western blotting and b) genetic comparison with FWPV and two previous isolates of poxvirus from Hawaiian forest birds, by i) restriction

[A]Present address: USDA-ARS Avian Disease and Oncology Laboratory, 3606 E. Mount Hope Road, East Lansing, MI 48823.

[B]Corresponding author.

The GenBank accession number of the nucleotide sequence reported in this paper is AY255628.

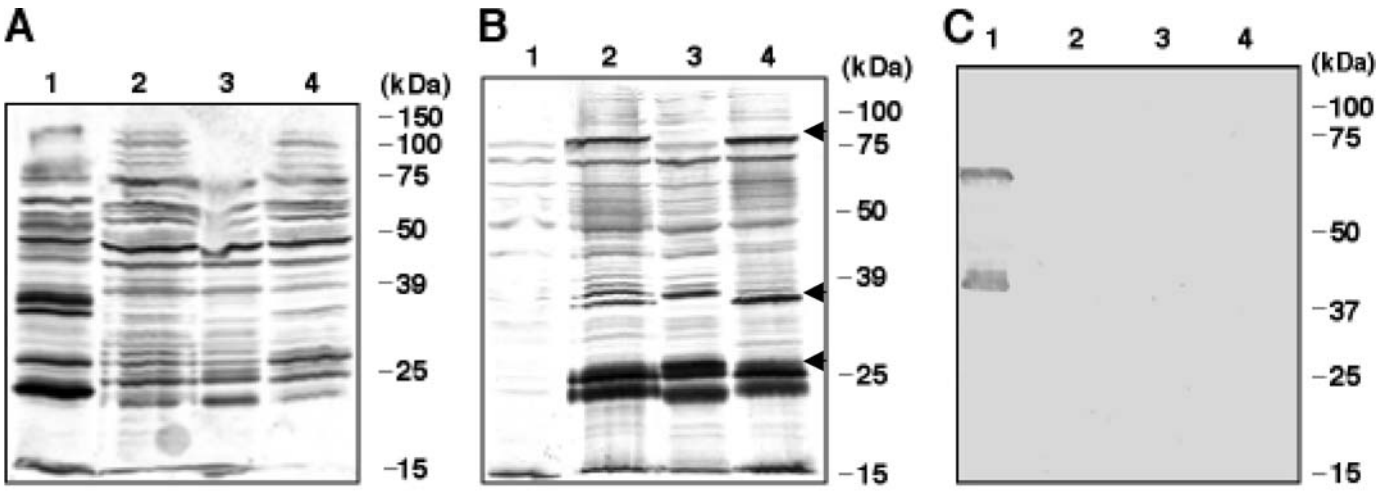

Fig. 1. Comparative Western blotting analysis of HGP isolate, two avian poxviruses previously isolated from Hawaiian forest birds (19), and FWPV antigens. Soluble viral proteins were separated by 12% SDS-PAGE and transferred onto PVDF. Viral antigens were detected by reaction with either (A) polyclonal anti-FWPV chicken serum or (B) polyclonal anti-Alala/lanakila isolate chicken serum or (C) FWPV-specific monoclonal antibodies (P1D9 and P2D4). The mobility of molecular weight markers (BioRad) is indicated on the right side of the blot. Lane 1: FWPV; lane 2: HGP isolate; lane 3: Apapane/4263-219 isolate; lane 4: Alala/lanakila isolate.

fragment length polymorphisms (RFLP) of their viral genomes, ii) polymerase chain reaction (PCR) amplification of specific sequences present in the genome of FWPV, iii) nucleotide sequence determination of a specific genomic fragment in HGP isolate and its comparison with other available avian poxvirus sequences.

MATERIALS AND METHODS

Isolation and propagation of virus. Cutaneous lesions suspected as poxvirus infection from an endangered Hawaiian forest bird, the state bird of Hawaii, Hawaiian goose (*Branta sandvicensis*) were received from Keauhou Conservation Center, HI. The lesions were ground with sterile alundum in Hanks balanced salt solution (Invitrogen, Carlsbad, CA) containing 1000 units penicillin, 1 mg streptomycin, and 2.5 μg amphotericin B per ml (17). After incubation at 37 C for 1 hr, the suspension was clarified by centrifugation at 1000 × *g* for 5 min at 4 C and the resulting supernatant inoculated onto chorioallantoic membranes (CAMs) of 10-day-old developing chicken embryos from specific-pathogen-free flock (SPAFAS, Norwich, CT). After incubation at 37 C for 7 days, CAMs were examined for pock lesions. Part of the CAM lesions were collected for virus isolation and part were fixed in 10% buffered formalin for histopathological examination as described earlier (19). A ground suspension of the CAM lesions was used as inocula for propagation of virus in permanent chicken liver tumor cells, LMH (9). Two avian poxviruses previously isolated from Hawaiian forest birds (Alala/lanakila and Apapane/4263-219 isolates) and a FWPV field isolate propagated in LMH cells were included for comparison.

Antigenic comparison of HGP isolate with FWPV and other Hawaiian poxvirus isolates. Viral antigens were isolated from LMH cells infected with either FWPV, HGP, or each of the two previous avian poxvirus isolates as previously described (8). Briefly, virus-infected cell monolayers were scraped and the cells were pelleted by centrifugation at 1500 × *g* for 10 min at 4 C, washed with isotonic buffer (10 mM Tris, pH 8.0, 150 mM NaCl, 5 mM EDTA) and lysed in 10 ml of hypotonic buffer (10 mM Tris, pH 8.0, 10 mM KCl, 5 mM EDTA) containing 0.025% β-mercaptoethanol and 0.1% triton X-100. After removing the cell nuclei by centrifugation at 1000 × *g* for 5 min at 4 C, the viral cores were pelleted by centrifugation at 11,000 × *g* for 90 min at 4 C. The resulting supernatants were used as a source of soluble viral antigens. The protein concentration of viral antigens was determined by BioRad protein assay reagent (BioRad, Hercules, CA) and then adjusted to 2 mg/ml before use. The antigens were resolved by SDS-PAGE and transferred onto polyvinylidinefluoride (PVDF) membrane (BioRad) (14). Nonspecific binding sites on the PVDF membrane were blocked with 3% bovine serum albumin (Sigma, St. Louis, MO) in phosphate-buffered saline (PBS, pH 7.4) at 37 C for 1 hr. After a wash with PBS containing 0.05% Tween 20 (Sigma) (PBST), polyclonal chicken serum (1 : 1500 dilution) raised against either FWPV or Alala/lanakila isolate was added separately and incubated for 2 hrs at 37 C. Following incubation and after three washes with PBST, alkaline phosphatase-conjugated anti-chicken immunoglobulin G (H+L) (1 : 2500 dilution) (Kirkegaard and Perry Laboratories, Gaithersburg, MD) was added and incubated for 1 hr at 37 C. After further washing steps, the secondary antibodies were detected by alkaline phosphatase conjugate substrate kit (BioRad) and the membrane were washed and dried.

Additionally, two FWPV-specific monoclonal antibodies (P1D9 and P2D4) (tissue culture supernatant of hybridomas producing monoclonal antibody) (12,14) were tested with antigens of HGP isolate. In this case, horseradish peroxidase-conjugated anti-mouse immunoglobulin G (H+L) (BioRad) (1 : 3000 dilution) was used for detection of the reactivity with monoclonalantibodies.

Isolation of viral DNA and comparison of RFLP. Viral DNA was extracted from the pelleted cores obtained from infected cell cultures described above by using DNAzol® reagent (Invitrogen) according to the manufacturer's instructions. To compare RFLP of genomes of avian poxviruses, each viral DNA was digested with *Hin*dIII (Invitrogen) and restriction digestion patterns were analyzed by electrophoresis at 60 volts for 16 hrs in 1% agarose gel at 4 C and staining with ethidium bromide.

PCR amplification of selected sequences of avian poxvirus and reticuloendotheliosis virus (REV). PCR amplifications were conducted using specific primers for amplification of a 1.2-kb region of FWPV open reading frame (ORF) 108 encoding a major antigenic envelope protein (1,5). Because all strains of FWPV contain either full-length reticuloendotheliosis virus (REV), provirus, or remnants of long terminal repeats (LTR) sequences, it was important to examine if HGP isolate had any of these sequences in its genome (12). For detection of the inserted REV LTR sequences, specific primers that amplify a 280 bp were used (5,11). With a view to determining the presence of REV provirus, primers for amplification of an 860-bp portion of the integrated REV envelope gene nucleotide sequence were derived from FWPV, UI (16) strain (5; GenBank accession number AF246698).

To amplify FWPV sequences that flank REV LTR remnant, degenerate primers that correspond to regions in FWPV ORF 201 and 203, respectively, flanking the REV integration site in the FWPV genome, were used (5). PCR mixture and conditions were as described earlier (5,6). The amplicons were analyzed by gel electrophoresis using 0.8% agarose and staining with ethidium bromide.

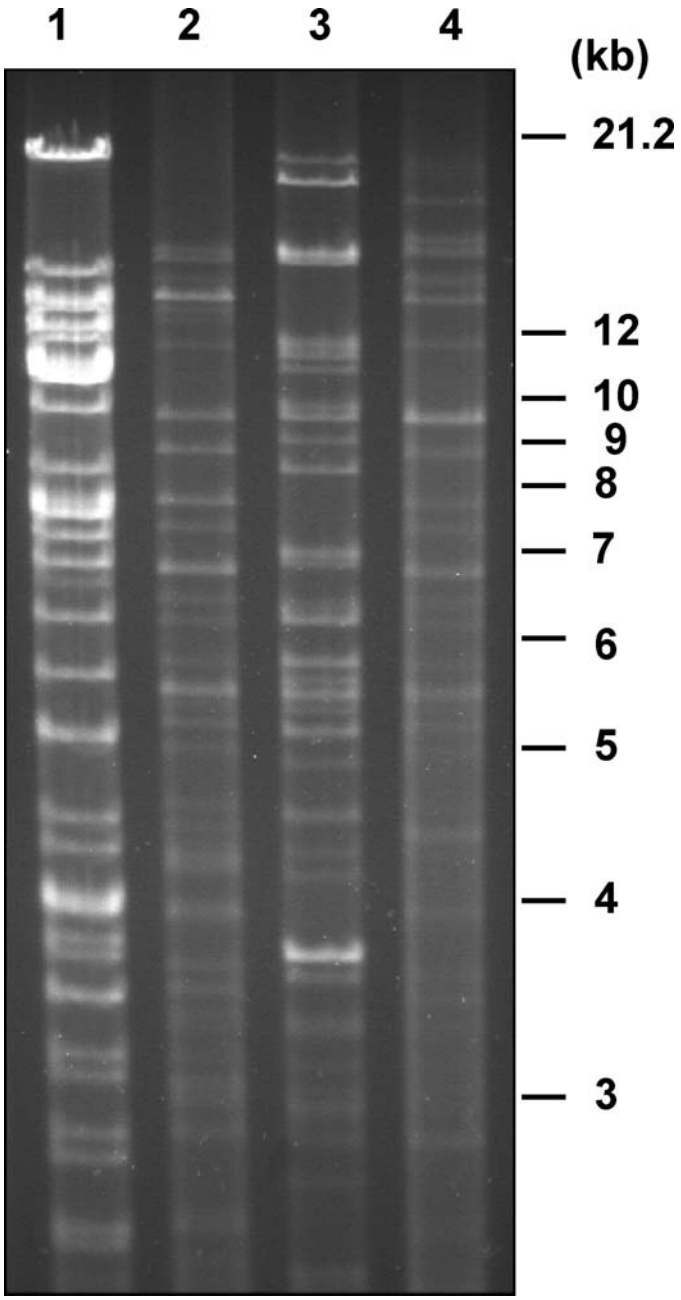

Fig. 2. Agarose gel electrophoresis of *Hin*dIII-generated fragments of the genomes of HGP isolate, two avian poxviruses previously isolated from Hawaiian forest birds and FWPV. The sizes of the fragments are shown on the right side of the figure. Lane 1: FWPV; lane 2: HGP isolate; lane 3: Apapane/4263-219 isolate; lane 4: Alala/lanakila isolate.

Cloning and nucleotide sequence determination of a 5.3-kb genomic fragment of HGP isolate and comparison with other avian poxviruses. Viral DNA of HGP isolate digested with restriction enzymes (*Pst*I and *Hin*dIII) (Invitrogen) was cloned into corresponding restriction sites of pUC19 plasmid. Recombinant clones were examined as described earlier (5,15). The nucleotide sequences of the terminal regions of selected HGP isolate genomic fragments were determined by using the universal forward/reverse primers and ABI PRISM® BigDye™ Terminator, version 3.0, Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA). A fragment of 5.3 kb was selected for determination of its complete sequence. To complete and verify the sequence of an approximately 5.3-kb fragment,

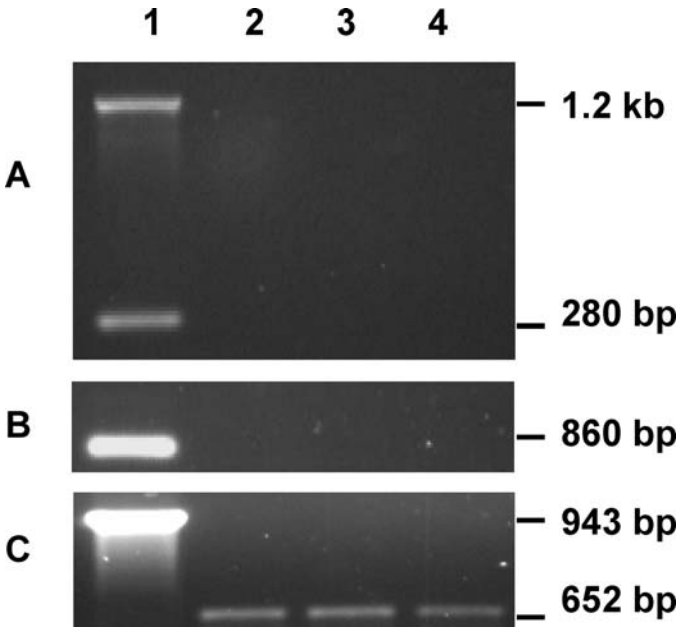

Fig. 3. Agarose gel electrophoresis of PCR products amplified from the genomes of HGP isolate, two avian poxviruses previously isolated from Hawaiian forest birds and FWPV. (A) FWPV major envelope antigen gene (1.2 kb) and REV 5′LTR (280 bp), (B) REV envelope gene (860 bp), (C) the flanking region amplification of integrated REV LTR. The sizes of the amplicons are shown on the right side of the figure. Lane 1: FWPV; lane 2: HGP isolate; lane 3: Apapane/4263-219 isolate; lane 4 Alala/lanakila isolate.

the internal region-specific primers were used. To determine the phylogenetic relationship of HGP isolate with fowl poxvirus (genus *Avipoxvirus*) and representatives of other genera in the Poxviridae family, nucleotide sequences of FWPV ORF197 (virion assembly protein gene; vaccinia virus A32L) homolog of other poxviruses (*Avipoxvirus*: fowl poxvirus, canary poxvirus, and *Vultur gryphus* poxvirus; *Leporipoxvirus*: myxoma virus and rabbit fibroma virus; *Molluscipoxvirus*: molluscum contagiosum virus; *Orthopoxvirus*: camel poxvirus, cow poxvirus, monkey poxvirus, vaccinia virus, and variola virus; *Suipoxvirus*: swine poxvirus; *Yatapoxvirus*: yaba monkey tumor virus) were aligned using Clustal W alignment tool and the phylogenetic tree was generated by the maximum-likelihood method using MegAlign software (DNASTAR Inc., Madison, WI). The nucleotide sequences were also compared with other avian poxvirus isolates counterpart using BL2SEQ (compare proteins to each other with BLAST) function at the Biology WorkBench website (workbench.sdsc.edu).

RESULTS

Propagation of virus into chicken embryo and avian cell culture. The causative pathogen produced marked thickenings on the CAMs of the developing chicken embryos following inoculation of ground suspension of cutaneous lesions from Hawaiian goose (not shown). Cytoplasmic inclusion bodies characteristic of poxvirus infection were observed in the hematoxylin and eosin-stained sections of virus-infected CAM lesions on histopathological examination (not shown). The virus initially isolated in the CAMs of developing chicken embryos could be easily adapted to LMH cells in which the cytopathic effect was characterized by plaque formation.

Antigenic characterization of HGP isolate. Although several cross reacting antigens were detected among the four avian poxviruses when polyclonal antibodies against FWPV or a previous Hawaiian isolate were used, the antigenic profile of HGP isolate was

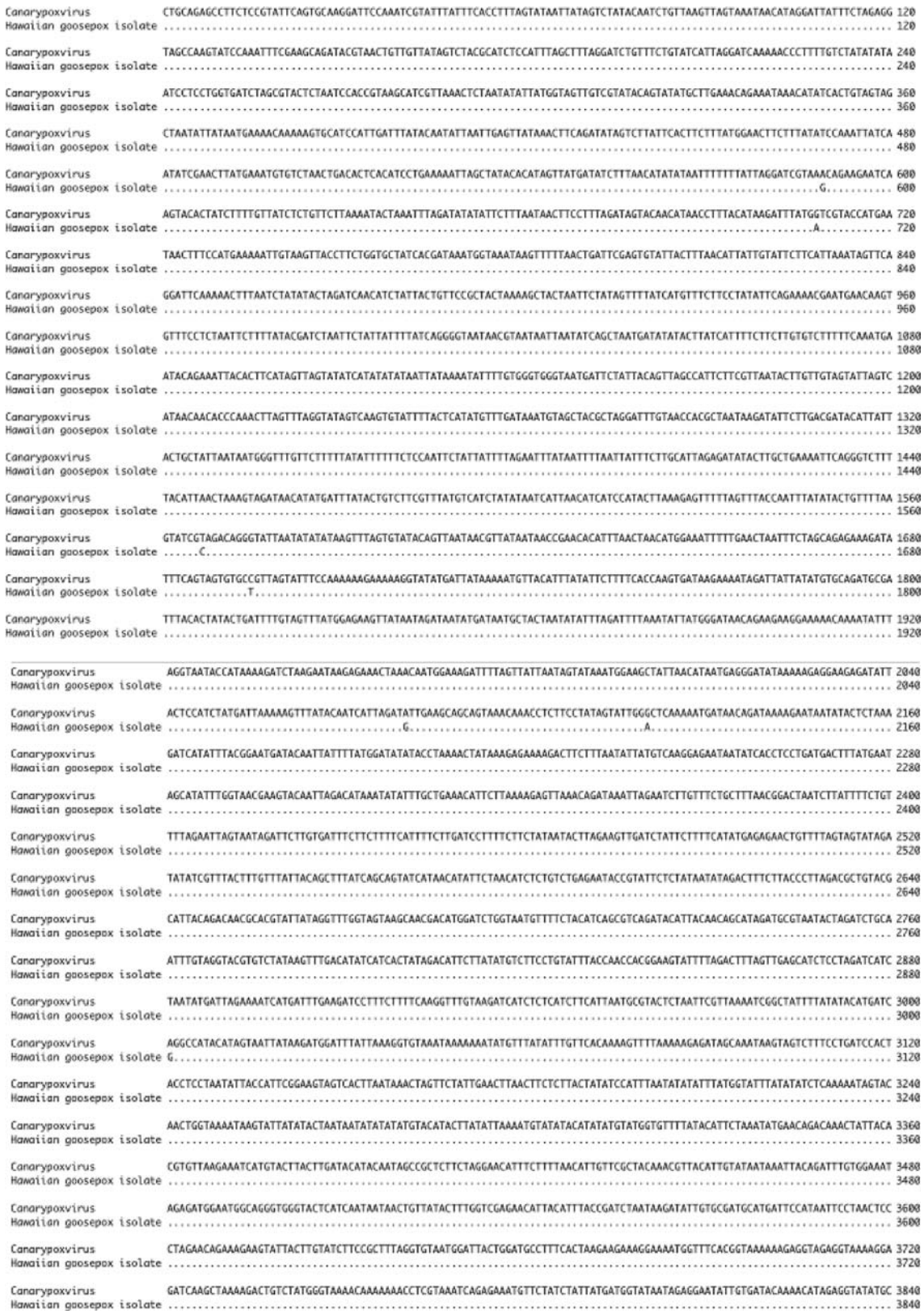

Fig. 4. Nucleotide sequence differences between a 5.3-kb fragment of HGP (AY255628) isolate and canary poxvirus (AY318871).

quite different with the respective antiserum (Fig. 1A,B). In this regard, antigenic profiles of two previous Hawaiian poxvirus isolates and HGP isolate appeared very similar when antibodies against FWPV were used. However, using antibodies against a previous Hawaiian poxvirus isolate (Alala/lanakila), minor differences by the presence or absence of antigens of approximately 27, 34, 35, and 81 kDa were observed (Fig. 1B). Monoclonal antibodies (P1D9 and P2D4) against FWPV didn't show any reaction with antigens of either HGP or two previous Hawaiian isolates (Apapane/4263-219 and Alala/lanakila) (Fig. 1C).

Fig. 4. Continued.

Comparison of restriction enzyme digestion patterns of HGP isolate genome with other avian poxviruses. With a view to determine genetic relationship of HGP isolate to two previous Hawaiian poxvirus isolates (Alala/lanakila, Apapane/4263-219) and FWPV, each viral genome was isolated and digested with *Hin*dIII. The generated DNA fragments were compared following electrophoresis in 1% agarose gel and staining with ethidium bromide. Although several comigrating fragments were detected among these viruses, the overall RFLP profile of HGP isolate is different from that of FWPV and Apapane/4263-219 isolate. However, the profile of Alala/lanakila isolate appears similar to HGP isolate (Fig. 2).

PCR amplification of selected avian poxvirus sequences in the genome of HGP isolate. Interestingly, FWPV-specific sequences of major envelope protein gene encoded by ORF 108 were not amplified in the genome of HGP isolate (Fig. 3A). Also, none of the REV sequences integrated in the FWPV genome (envelope and LTR remnant sequences present in FWPV) were amplified in its genome (Fig. 3A,B). However, using degenerate primers to amplify sequences that flank the REV integration site, a smaller size fragment of 652 bp was generated in the genome of HGP isolate and two previous Hawaiian poxviruses compared with that of 943 bp in FWPV (Fig. 3C). This size difference in PCR product of FWPV is because of amplification of flanking REV LTR sequences during PCR amplification.

Comparison of the nucleotide sequences of HGP isolate genomic fragment with other avipoxviruses. The nucleotide sequences of a 5.3-kb *Pst*I-*Hin*dIII fragment of the genome of HGP isolate showed 99% identities with canary poxvirus (CNPV) ORF266-274 (20) (Fig. 4). A similar size fragment with high homology to CNPV sequence was observed in VGPV isolated from Andean condor (5). Like CNPV and VGPV, this fragment did not contain FWPV homologs of ORFs 199, 200, and 202. While

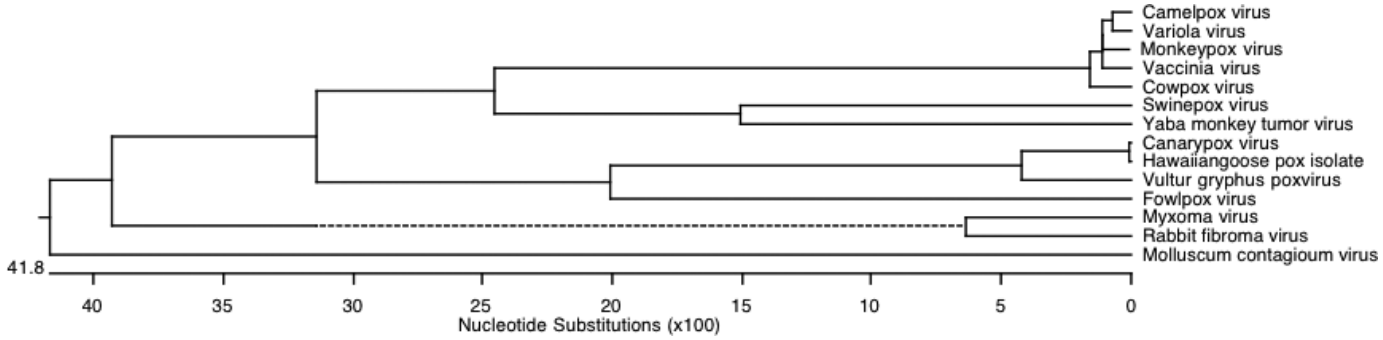

Fig. 5. Phylogenetic comparison of virion assembly protein gene homolog (FWPV ORF197) in poxviruses. The amino acid sequences were aligned by using the Clustal W program and a phylogenetic tree was generated by the maximum-likelihood method. Accession number of nucleotide sequences is as follow: fowl poxvirus (AF198100), canary poxvirus (AY318871), *Vultur gryphus* poxvirus (AY246559), Hawaiian goosepox isolate (AY255628), *Myxoma* virus (AF170726), rabbit fibroma virus (AF170722), *Molluscum contagiosum* virus (U86943), camel poxvirus (AY009089), cow poxvirus (AF482758), monkey poxvirus (AF380138), vaccinia virus (AY243312), variola virus (NC001611), swine poxvirus (AF410153), Yaba monkey tumor virus (AB025319).

Table 1. Comparison of a 5.3-kb *Pst*I-*Hin*dIII fragment of HGP isolate genomic DNA (ORFs 193, 194, 195, 196, 197, 198, 201) with corresponding region of CNPV, VGPV, and FWPV genome. The positives of the predicted amino acid sequences among HGP isolate, CNPV, VGPV, and FWPV encoded proteins are shown. The predicted functions of ORFs products are indicated. The GenBank accession numbers of each virus nucleotide sequences are as follow: FWPV (AF198100), VGPV (AY246559), CNPV (AY318871), and HGP isolate (AY255628).

Positives	VGPV	HGP	CNPV
ORF193 (RNA polymerase subunit)			
FWPV	85%	80%	80%
VGPV		99%	99%
HGP			99%
ORF194 (unknown)			
FWPV	90%	94%	90%
VGPV		98%	98%
HGP			100%
ORF195 (unknown)			
FWPV	84%	85%	86%
VGPV		97%	99%
HGP			99%
ORF196 (unknown)			
FWPV	66%	63%	64%
VGPV		93%	93%
HGP			99%
ORF197 (virion assembly protein)			
FWPV	86%	84%	84%
VGPV		96%	96%
HGP			100%
ORF198 (C-type lectin-like protein)			
FWPV	78%	69%	79%
VGPV		93%	94%
HGP			100%
ORF201 (unknown)			
FWPV	68%	68%	69%
VGPV		93%	93%
HGP			100%

the similarity of predicted amino acid sequences of the encoded proteins was 63–94% with FWPV, it was 93–99% with VGPV, and 99–100% with CNPV counterparts (Table 1).

Phylogenetic comparison of the nucleotide sequences of FWPV ORF197 homolog (virion assembly proteins gene) of other poxviruses (*Avipoxvirus*, *Leporipoxvirus*, *Molluscipoxvirus*, *Orthopoxvirus*, *Suipoxvirus* genus) confirmed that the HGP isolate belongs to *Avipoxvirus* genus (Fig. 5).

DISCUSSION

Poxvirus infection in avian species is one of wide host–range animal viral diseases. Approximately 232 species in 23 orders of birds have been reported to acquire a natural poxvirus infection (2). With the exception of FWPV, the type species of *Avipoxvirus* genus, very limited antigenic, genetic, and biological information toward characterization of avian poxviruses is available. While complete nucleotide sequence of fowl poxvirus and canary poxvirus genomes has been determined recently, genetic information on other avian poxviruses is not available at this time. In this regard, the genome of a vaccine-like strain of FWPV contained only REV LTR sequences in its genome. In contrast, most of the field isolates of FWPV have REV provirus sequences in their genome. Thus, for characterization of field isolates, especially from wild birds, combination of genetic analysis of viral genome, immunological evaluation of viral antigens, and cross-protection studies are necessary to differentiate the isolates (18).

In this study, a novel avian poxvirus isolated from an endangered Hawaiian goose, which is the state bird of Hawaii, was characterized genetically by RFLP of viral genome, PCR amplification of specific genomic sequences, nucleotide sequence determination of genomic fragment, and antigenically by Western blot analysis using antibodies against avian poxviruses. FWPV and two previous avian poxvirus isolates from Hawaiian forest birds were included for this comparison. Although, often field isolates of avian poxviruses, especially from wild birds, fail to grow in cell cultures (5,19), the characterization of HGP isolate was possible because of ease of its adaptation in LMH cells.

Because the presence of REV sequences in FWPV genome determines a unique evolutionary event, which occurred over 50 yr ago (5,6,11,13), examination of this genetic marker has become important to differentiate FWPV from other *Avipoxvirus* isolates. In this regard, all field strains of FWPV reveal REV provirus integration in their genome while the vaccine strains of FWPV contain LTR remnants of REV (13). Interestingly, a poxvirus isolated from a wild turkey (Kim and Tripathy, unpubl. data) revealed integration of REV provirus in its genome, indicating its similarity with field strain of FWPV. On the other hand, the genome of a poxvirus isolated from ostriches lacked REV provirus sequences and had only REV LTR sequences in its genome (10). It revealed highly antigenic, genetic, and biological similarities to FWPV. The ostriches had been raised on premises where turkeys had been kept previously and evidence of poxvirus infection in the turkeys had been observed. In this case, the virus was considered as an FWPV vaccine because the vaccine is routinely used in chickens and turkeys for prevention of the disease in areas where the disease is endemic.

Although, Western blot analysis of HGP isolate shows its difference from FWPV, detection of cross-reacting antigens among the avian poxvirus isolates from Hawaiian birds (alala and apapane) indicates that many genes are conserved among these viruses. Detailed genetic and biological studies will be necessary to determine differences among these viruses.

Surprisingly, the nucleotide sequences of *Pst*I-*Hin*dII fragment of HGP isolates are very similar to canary poxvirus. Only eight nucleotides showed polymorphism in the 5.3-kbp region. From this comparison, it appears that HGP isolate is genetically more closely related to CNPV than FWPV. However, lack of a complete genomic sequence of HGP isolate hinders further genetic comparison of these two viruses. Phylogenetic comparison of virion assembly protein gene (FWPV ORF197) homolog confirmed that HGP isolate is a member of *Avipoxvirus* genus and is genetically more closely related to canary poxvirus than fowl poxvirus.

Results of this study clearly indicate that HGP isolate is genetically and antigenically different from type species of *Avipoxvirus*, FWPV as indicated by absence of FWPV specific major envelope antigen gene as well as lack of REV sequences in its genome using PCR amplification. Also, the heterogeneity is supported by major differences in its RFLP pattern, the absence of three FWPV corresponding ORFs in its genome, and lack of cross-reactivity with two FWPV specific monoclonal antibodies.

REFERENCES

1. Afonso, C. L., E. R. Tulman, Z. Lu, L. Zsak, G. F. Kutish, and D. L. Rock. The genome of fowlpox virus. J. Virol. 74:3815–3831. 2000.

2. Bolte, A. L., J. Meurer, and E. F. Kaleta. Avian host spectrum of avipoxviruses. Avian Pathol. 28:415–432. 1999.

3. Friend, M., and J. C. Franson. Avian pox. In: Field manual of wildlife diseases: general field procedures and diseases of birds. E. A. Ciganovich, ed. U.S. Department of the Interior and U.S. Geological Survey, Washington, DC. pp. 163–169. 1999.

4. Ghildyal, N., W. M. Schnitzlein, and D. N. Tripathy. Genetic and antigenic differences between fowlpox and quailpox viruses. Arch. Virol. 106: 85–92. 1989.

5. Kim, T.-J., W. M. Schnitzlein, D. McAloose, A. P. Pessier, and D. N. Tripathy. Characterization of an avianpox virus isolated from an Andean condor (*Vultur gryphus*). Vet. Micro. 96:237–246. 2003.

6. Kim, T.-J., and D. N. Tripathy. Reticuloendotheliosis virus integration in the fowl poxvirus genome: not a recent event. Avian Dis. 45:663–669. 2001.

7. Moyer, R. W., B. M. Arif, D. N. Black, D. B. Boyle, R. M. Buller, K. R. Dumbell, J. J. Esposito, G. McFadden, B. Moss, A. A. Mercer, S. Ropp, D. N. Tripathy, and C. Upton. Family *Poxviridae*. In: Virus taxonomy: classification and nomenclature of viruses, 7th report of the International Committee on Taxonomy of Viruses. Academic Press, San Diego, CA. pp.137–157. 2000.

8. Schnitzlein, W. M., N. Ghildyal, and D. N. Tripathy. Genomic and antigenic characterization of avipoxviruses. Virus Res. 10:65–76. 1988.

9. Schnitzlein, W. M., J. Radzevicius, and D. N. Tripathy. Propagation of infectious laryngotracheitis virus in an avian liver cell line. Avian Dis. 38: 211–217. 1995.

10. Shivaprasad, H. L., T.-J. Kim, P. R. Woolcock, and D. N. Tripathy. Genetic and antigenic characterization of a poxvirus isolate from ostriches. Avian Dis. 46:429–436. 2002.

11. Singh, P., T.-J. Kim, and D. N. Tripathy. Re-emerging fowlpox: evaluation of isolates from vaccinated flocks. Avian Pathol. 29:449–455. 2000.

12. Singh, P., T.-J. Kim, and D. N. Tripathy. Identification and characterization of fowlpox virus strains utilizing monoclonal antibodies. J. Vet. Diagn. Invest. 15:50–54. 2003.

13. Singh, P., W. M. Schnitzlein, and D. N. Tripathy. Reticuloendotheliosis virus sequences within the genomes of field strains of fowlpox virus display variability. J. Virol. 77:5855–5862. 2003.

14. Singh, P., and D. N. Tripathy. Characterization of monoclonal antibodies against fowl poxvirus. Avian Dis. 44:365–371. 2000.

15. Srinivasan, V., W. M. Schnitzlein, and D. N. Tripathy. Fowlpox virus encodes a novel DNA repair enzyme, CPD-photolyase, that restores infectivity of UV light-damaged virus. J. Virol. 75:1681–1688. 2001.

16. Tripathy, D. N., L. E. Hanson, and A. H. Killinger. Atypical fowlpox in a poultry farm in Illinois. Avian Dis. 18:84–90. 1974.

17. Tripathy, D. N., and W. M. Reed. Pox. In: A laboratory manual for the isolation and identification of avian pathogens, 4th ed. D. E. Swayne, J. R. Glisson, M. W. Jackwood, J. E. Pearson, and W. M. Reed, eds. American Association of Avian Pathologist, University of Pennsylvania, Kennet Square, PA. pp. 137–140. 1998.

18. Tripathy, D. N., and W. M. Reed. Pox. In: Diseases of poultry, 11th ed. Y. M. Saif, H. J. Barnes, B. W. Calnek, J. R. Glisson, A. M. Fadly, L. R. McDougald, and D. E. Swayne, eds. Iowa State University Press, Ames, IA. pp. 253–269. 2003.

19. Tripathy, D. N., W. M. Schnitzlein, P. J. Morris, D. L. Janssen, J. K. Zuba, G. Massey, and C. T. Atkinson. Characterization of poxviruses from forest birds in Hawaii. J. Wildl. Dis. 36:225–230. 2000.

20. Tulman, E. R., C. L. Afonso, Z. Lu, G. F. Kutish, and D. L. Rock. The genome of canarypox virus. J. Virol. 78:353–366. 2004.

21. Warner, R. E. The role of introduced diseases in the extinction of the endemic Hawaiian avifauna. Condor 70:101–120. 1968.

ACKNOWLEDGMENTS

The authors thank Dr. William M. Schnitzlein for his technical support to accomplish this work. The help of Dr. Pat Morris, San Diego Zoo, and Mr. Alan Liebermann, Keauhou Conservation Center, Hawaii, is gratefully acknowledged. This work was supported by a grant from Morris Animal Foundation and Regional Research Funds of the University of Illinois.